PB86-141264

Methods for Evaluating the Biological Impact of Potentially Toxic Waste Applied to Soils

Cornell Univ., Ithaca, NY

Prepared for

Corvallis Environmental Research Lab., OR

Dec 85

U.S. Department of Commerce
National Technical Information Service
NTIS

PB86-141264

EPA/600/D-84/298
December 1985

Methods for Evaluating the Biological Impact of Potentially Toxic Waste Applied to Soils

by

Edward F. Neuhauser, Raymond C. Loehr, and
Michael R. Malecki

Riley-Robb Hall
Cornell University
Ithaca, NY 14853

EPA Cooperative Agreement
CR-810006

EPA Project Officer
Clarence A. Callahan

ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CORVALLIS, OR 97333

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA/600/D-84/298	2.
3. RECIPIENT'S ACCESSION NO. PB86 141264/AS	
4. TITLE AND SUBTITLE Methods for Evaluating the Biological Impact of Potentially Toxic Waste Applied to Soils	5. REPORT DATE December 1985
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Edward F. Neuhauser, Raymond C. Loehr and Michael R. Malecki	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Riley-Robb Hall, Cornell University, Ithaca, New York 14853	10. PROGRAM ELEMENT NO.
	11. CONTRACT/GRANT NO. CR-810006
12. SPONSORING AGENCY NAME AND ADDRESS Environmental Research Laboratory, Office of Research and Development, 200 SW 35th Street, Corvallis, Oregon 97333	13. TYPE OF REPORT AND PERIOD COVERED Journal Article
	14. SPONSORING AGENCY CODE EPA/600-02

15. SUPPLEMENTARY NOTES

16. ABSTRACT

This study was designed to evaluate two methods that can be used to estimate the biological impact of organics and inorganics that may be in wastes applied to land for treatment and disposal. The two methods were the contact test and the artificial soil test. The contact test is a 48 hr test using an adult worm, a small glass vial, and filter paper to which the test chemical or waste is applied. The test is designed to provide close contact between the worm and a chemical similar to the situation in soils. The method provides a rapid estimate of the relative toxicity of chemicals and industrial wastes. The artificial soil test uses a mixture of sand, kaolin, peat and calcium carbonate as a representative soil. Different concentrations of the test material are added to the artificial soil, adult worms are added and worm survival is evaluated after two weeks.

These studies have shown that: (a) earthworms can distinguish between a wide variety of chemicals with a high degree of accuracy, (b) earthworms are a suitable biomonitoring tool to measure the impact of chemicals in wastes added to soils, (c) the contact and artificial soil tests can measure the biological impact of chemicals applied to soils, (d) earthworms can differentiate the impact of chemical groups and specific chemicals, and (e) the contact and artificial soil tests appear to identify the same relative toxicity of chemicals as rat toxicity data.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT Release to Public	19. SECURITY CLASS *(This Report)* Unclassified	21. NO. OF PAGES 26
	20. SECURITY CLASS *(This page)* Unclassified	22. PRICE

EPA Form 2220-1 (9-73)

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

ABSTRACT

This study was designed to evaluate two methods that can be used to estimate the biological impact of organics and inorganics that may be in wastes applied to land for treatment and disposal. The two methods were the contact test and the artificial soil test. The contact test is a 48 hr test using an adult worm, a small glass vial, and filter paper to which the test chemical or waste is applied. The test is designed to provide close contact between the worm and a chemical similar to the situation in soils. The method provides a rapid estimate of the relative toxicity of chemicals and industrial wastes. The artificial soil test uses a mixture of sand, kaolin, peat and calcium carbonate as a representative soil. Different concentrations of the test material are added to the artificial soil, adult worms are added and worm survival is evaluated after two weeks.

These studies have shown that: (a) earthworms can distinguish between a wide variety of chemicals with a high degree of accuracy, (b) earthworms are a suitable biomonitoring tool to measure the impact of chemicals in wastes added to soils, (c) the contact and artificial soil tests can measure the biological impact of chemicals applied to soils, (d) earthworms can differentiate the impact of chemical groups and specific chemicals, and (e) the contact and artificial soil tests appear to identify the same relative toxicity of chemicals as rat toxicity data.

Keywords: land treatment, contact test, artificial soil test, industrial wastes, earthworms, biological testing.

INTRODUCTION

Land application has been used successfully to treat many industrial wastes. However, laboratory and field studies of this treatment method rarely consider the impact of potentially hazardous waste materials on the soil ecosystem. This paper evaluates methods that measure the impact of waste materials on earthworms, an important component of the soil biota. Previous work with this methodology was reported at an earlier ASTM Symposium (Neuhauser et al., 1983).

The complexity of soil fauna is a complicating factor when biological assays are used to evaluate the impact of wastes on the soil ecosystem. Soil microorganisms and soil arthropods are very diverse, are difficult to identify and are difficult to use in biological testing procedures. Earthworms are a major biological component of soils, are relatively easy to obtain in large numbers and biomass and can be a suitable soil test organism.

This paper discusses the applicability of two methods originally developed by the European Economic Community (EEC 1982) to estimate the biological impact of wastes applied to soils. These methods are designed to evaluate the short-term impact of materials on earthworm survival through skin absorption (contact test) and the longer term impact on earthworm survival through skin absorption and ingestion (artificial soil test).

PROCEDURES

Earthworms - Eisenia fetida was the earthworm species used in these test methods. Large numbers of this species of known age and size can be obtained easily for testing. Although E. fetida occurs in specific habitats in temperate climates, considerable information has accumulated concerning the growth (Neuhauser et al., 1980), reproduction (Hartenstein et al., 1979), physical requirements (Kaplan et al., 1980), use in waste management systems (Neuhauser et al., 1980), and the response of the worms to metals (Malecki et al., 1982 and Neuhauser et al., 1983). This body of information and the ease of using this species under laboratory conditions has made it appropriate to use E. fetida in biological testing methods.

The worms used in these experiments were obtained from stock cultures in the laboratory of the authors. The cultures are maintained at room temperature (21 to 24°C), with cocoons being harvested every two weeks. Aerobically digested domestic sludge was used as the maintenance organic food substrate for the worms.

Chemicals - Both organic and inorganic chemicals were used to evaluate the potential impact of wastes applied to soils. The water soluble salts (acetate, chloride, nitrate and sulfate) of five metals (Cd, Cu, Ni, Pb and Zn) were selected on the basis of their potential toxicity and presence in municipal and industrial wastes that may be applied to soils. All of the metal salts used were Fisher certified chemicals. The organic chemicals used in these studies were organic priority pollutants (EPA 1979) and were compounds as pure as could be obtained.

Contact Test - The contact test procedures described by the EEC (1982) were followed. In this test, glass vials are lined with filter paper to

which the chemical is added. The chemical is distributed over the surface of the paper, one worm is added to each vial and the vials are incubated at 20°C. The worm is in contact with the chemical and can absorb the chemical through its skin. After incubation for a defined time period (two days at 20°C), worm survival is determined. A range of chemical concentrations is evaluated and a large number of replicates are used for each concentration. The results are evaluated to determine a LC_{50} value for each chemical.

Glass vials (3 oz) were lined with 12 x 6.5 cm of filter paper to give an area of 80 cm^2 (Fig. 1). Fresh aqueous metal salt solutions were made for each series of tests.

The testing of organic chemicals used distilled water as the solvent if possible. If the test chemical was not soluble in water, other solvents (acetone or chloroform) were used. The organic solvent used to add the chemical should volatilize readily and be evaporated from the vial before the one mℓ of distilled water and the worm are added to the vial. The loss of organic solvents was evaluated and did occur in all cases before the worms were added. In addition, experimentation determined that none of the chemicals added to the vials with the organic solvents volatilized with the organic solvent. The one mℓ of water was added to provide enough moisture for earthworm survival. One adult earthworm (300-500 mg) was added per vial and the vials were kept on their side at 20°C in a darkened incubator. Only one worm is added per vial because the death of one worm in the vial could adversely affect the well-being of any other worms present.

After 48 hours, the viability of the earthworms was determined. Earthworms were considered dead if they did not respond to a gentle mechanical

CONTACT TEST

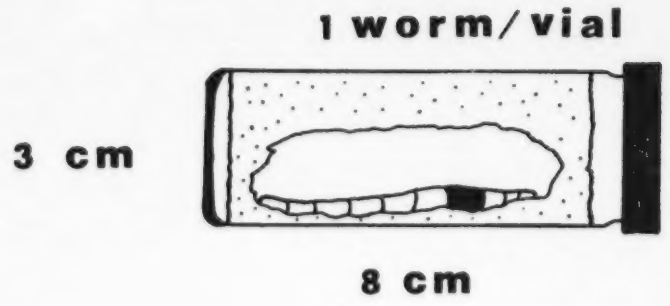


Figure 1. Cut away view of glass vial lined with filter paper, 12 cm x 6.7 cm = 80 cm^2 per vial.

stimulus. The number of dead worms was related to the concentration of the chemical to which the worms were exposed.

The LC_{50} value for each chemical tested was obtained by first using a series of screening tests to determine the range of chemical concentrations that had an adverse impact. This permitted the use of a narrow, more definitive range to determine the more specific LC_{50} value. The range finding tests included concentrations that resulted in mortalities from zero to 100 percent. This was accomplished by using chemical concentrations that varied over several logs.

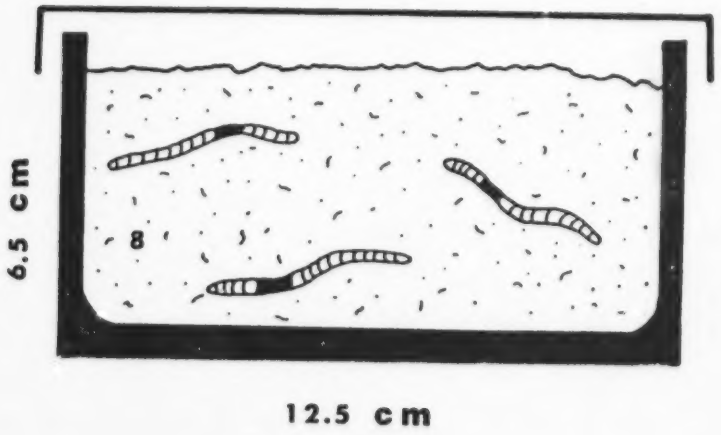
At least five concentrations were evaluated in the definitive test, with ten or more replicates included for each concentration tested. Controls, which included vials that contained everything except the test chemical, were included in each series of experiments.

The LC_{50} value for each chemical tested was calculated using the method of Litchfield and Wilcoxon (1949). In the contact test, the LC_{50} values are reported in terms of mg of test chemical per cm^2 of filter paper.

Artificial Soil Test - The EEC tentative artificial soil test method (EEC 1982) was followed exactly. This test uses a medium which consists by weight of 10% finely ground sphagnum peat, 20% colloidal kaolin clay, 69% fine sand and 1% pulverized calcium carbonate. The final pH was adjusted to 6.0 $\pm$ 0.5. The water content was raised to 35% of the dry weight of the four components. The test chemical was dissolved in water and then added to the artificial soil.

If the test chemical was not water soluble, it was mixed with a small volume of suitable organic solvent (acetone or chloroform) and then mixed with the artificial soil. Tests were run to determine the amount of time needed to completely evaporate the organic solvent from the artificial soil. After the organic solvent evaporated, the artificial soil was brought to a moisture

ARTIFICIAL SOIL TEST



400 g/dish

69 % Industrial sand
20 % Kaolin clay
10 % Sphagnum peat
1 % Calcium carbonate

Figure 2. Cut away view of the set-up used for the 14 day artificial soil test at 20°C.

content of 35%. Controls, which consisted of all components except the test chemical, were included in each set of experiments.

Ten adult E. fetida (300-500 mg) were added to 400 g (dry weight) of the test mixture in round glass dishes 6.5 cm high and 12.5 cm in diameter (Fig. 2). The dishes were placed in an incubator at 20°C and mortality was assessed after two weeks. Earthworms were considered dead when they did not respond to a gentle mechanical stimulus.

Concentrations causing mortalities of zero to 100% were determined by using range finding tests that included chemical concentrations that varied over several logs. After the range of chemicals had been narrowed, a definitive test was performed. This test had at least five chemical concentrations, with four repetitions for each test concentration and four control dishes, all containing 10 worms per dish.

The LC_{50} value for each chemical was calculated using the method of Litchfield and Wilcoxon (1949) and is reported in terms of mg chemical per kg dry weight of artificial soil.

RESULTS

Metals - Four salts of each metal were used in the contact test to determine if the metal salts exhibited different toxicities (Table 1). Statistical analyses determined that the four salts of each metal (acetate, chloride, nitrate and sulfate) had similar LC_{50} values. The different water soluble salts of each metal did not show any difference in toxicity to the worms. Of the metals tested, copper (Cu) was the most toxic, followed by Zn, Ni, Cd and Pb in order of decreasing toxicity.

Table 1. The earthworm contact test values for the metals tested.

	Metal Salt, LC_{50}, 95% CI , $\mu g \cdot cm^{-2}$			
Metal	Acetate	Chloride	Nitrate	Sulfate
Cadmium	20(16-25)[a1]	18(12-25)[a]	24(12-45)[a]	26(17-39)[a]
Copper	6.7(5.3-8.5)[b]	4.9(4.0-5.9)[b]	7.4(3.8-14.4)[b]	6.3(4.6-8.6)[b]
Lead	50(30-83)[c]	NT[2]	64(45-92)[c]	NT
Nickel	17(13-22)[d]	17(12-24)[d]	25(19-34)[d]	20(16-25)[d]
Zinc	13(10-16)[f]	12(6-24)[f]	10(8-12)[f]	13(7-26)[f]

1. Means with a common letter are not significantly different, $p < 0.05$
2. NT - not tested

The artificial soil test requires more work than the contact test. Because none of the metal salts showed any differences, only one salt (nitrate) was used in the artificial soil test. The LC_{50} values obtained for the nitrate salts of the five metals tested in the artificial soil test are noted in Table 2.

The artificial soil test results also indicated that Cu, Ni and Zn were the most toxic metals with no statistical difference between these three metals. Cadmium was the next most toxic metal tested followed by Pb.

Organics - The results of the 44 organics tested with the earthworm contact test are summarized in Table 3. The phenols were the most toxic chemical group tested, followed closely by the amines. The major remaining chemical groups, in order of toxicity from most toxic to least toxic, were substituted aromatics, halogenated aliphatics, polycyclic aromatic hydrocarbons and phthalates.

The individual LC_{50} values for one of the chemical groups studied, the substituted aromatics, are summarized in Table 4. There was no statistical difference in toxicity between carbaryl and nitrobenzene, the most toxic substituted aromatics tested. The chlorine-substituted aromatics were all more toxic than the alkyl-substituted aromatics. Hexachlorobenzene, perhaps due to its extremely low solubility, 0.1 $\mu g \cdot ml^{-1}$, was the least toxic of all the substituted aromatics tested. The two day contact test may not have permitted enough time for the worms to be affected by the hexachlorobenzene.

The large amount of effort involved in conducting the artificial soil tests limited the number of chemicals that could be evaluated. Table 5 lists the LC_{50} values found for the organics tested with the artificial soil test in order of decreasing toxicity. The contact test LC_{50} values for the

Table 2. The artificial soil test values for the nitrate salts of the metals indicated.

Metal	Artificial Soil Test LC_{50} $mg \cdot kg^{-1}$, 95% CI
Cd	1843 (1660-2045)
Cu	643 (549 - 753)
Pb	5941 (5292-6670)
Ni	757 (661 - 867)
Zn	662 (574 - 674)

Table 3. Summary of the means and ranges of the LC_{50} values for the six chemical groups tested with the contact test.

Chemical Group	Worm LC_{50} $\mu g \cdot cm^{-2}$, Range	Rat, Oral, LD_{50} $mg \cdot kg^{-1}$, Range
Phenols (n=9)	2.9 (0.6 - 5.9)	1,000 (30 - 3,200)
Amines (n=3)	5.0 (2.0 - 11.0)	740 (70 - 1,650)
Substituted Aromatics (n=9)	41 (14 - 98)	2,100 (250 - 5,000)
Halogenated Aliphatics (n=14)	93 (10 - 304)	2,300 (75 - 10,000)
Polycyclic Aromatic Hydrocarbons (n=4)	1,470 (49-4,670)	4,560 (1,780 - 10,000)
Phthalates (n=5)	1,760 (550-3,140)	20,225 (6,900 - 31,000)

n= the number of chemicals tested with the contact test in the study.
test in the study.

Table 4. Earthworm LC_{50} contact test values for the substituted aromatics tested.

Chemical	LC_{50} $\mu g \cdot cm^{-2}$, 95% CI
Carbaryl	14 (10 - 19)[a1]
Nitrobenzene	16 (15 - 17)[a]
1,2 - Dichlorobenzene	21 (19 - 23)[b]
1, 2, 4 - Trichlorobenzene	27 (24 - 32)[c]
Chlorobenzene	29 (22 - 38)[c]
Ethylbenzene	47 (42 - 53)[d]
Toluene	75 (68 - 83)[f]
Benzene	98 (78-123)[f]
Hexachlorobenzene	non-toxic up to 1,000 $\mu g \cdot cm^{-2}$

Means with a common letter are not statistically different, $p < 0.05$.

Table 5. Impact of the chemicals in the artificial soil test and the contact test.

Chemical	Artificial Soil Test LC_{50}, $mg \cdot kg^{-1}$, 95% CI		Contact Test LC_{50}, $ug \cdot cm^{-2}$, 95% CI	
Chloroacetamide	26	(22 - 31)	2.0	(1.9 2.1)
4-Nitrophenol	38	(32 - 45)	0.7	(0.6 - 0.8)
2,4,6-Trichlorophenol	58	(54 - 62)	5.0	(4.1 - 6.1)
Carbaryl	106	(81 - 138)	14	(10 - 19)
N-Nitrosodiphenyl-amine	151	(136 - 167)	2.4	(2.2 - 2.6)
Fluorene	173	(150 - 201)	171	(128 - 224)
Phenol	401	(347 - 463)	5.0	(4.2 - 5.8)
2-Chloroethyl vinyl ether	740	(570 - 970)	33	(31 - 36)
Dimethyl phthalate	3,160	(2,450 3,920)	550	(440 - 690)
1,2-Dichloropropane	4,240	(3,830-4,680)	64	(59 - 70)

organics tested with the artificial soil test are included for comparative purposes. In general, the chemicals that were the most toxic to the worms in the contact test were the most toxic in the artificial soil test.

DISCUSSION

The development of any bioassay or toxicity index requires that the data obtained with the new methods be compared to existing toxicity data. One of the most readily available toxicity data bases is rat oral LD_{50} data. Table 6 lists: (a) the mean earthworm LC_{50} values and ranges, and (b) the mean rat oral LD_{50} values and range for the six chemical classes tested. The two chemical groups that were the most toxic to the earthworms, phenols and amines, were also the most toxic to the rat. The third most toxic group to the earthworms, substituted aromatics, was also the third most toxic chemical group to the rat. The other three chemical groups, halogenated aliphatics, polycyclic aromatic hydrocarbons and phthalates, were less toxic to the earthworms and the rats but had the same relative toxicity. This comparison suggests that chemicals toxic to the earthworms have the same relative toxicity to rats and vice versa.

Comparable data for specific organic chemicals, in terms of earthworm LC_{50} and rat LD_{50} values, are presented in Table 7. This comparison also shows the same relative toxicity pattern. The substituted aromatics that were the most toxic to the rat also were the most toxic to the worms. Carbaryl, nitrobenzene and 1,2 dichlorobenzene were the three most toxic substituted aromatics and toluene, benzene and hexachlorobenzene were the three least toxic substituted aromatics tested.

The LC_{50} data from the artificial soil test also were compared to the rat LD_{50} data (Table 8). Again, in general, the chemicals that were most toxic to the worms also were the most toxic to the rat.

Table 6. Summary of the means and ranges for the six chemical groups in the contact test and the means and ranges for the rat, oral, LD_{50} values for the same chemical groups.

Chemical Group	Worm LC_{50} $\mu g \cdot cm^{-2}$, Range
Phenols (n=9)	2.9 (0.6 - 5.9)
Amines (n=3)	5.0 (2.0 - 11.0)
Substituted Aromatics (n=9)	41 (14 - 98)
Halogenated Aliphatics (n=14)	93 (10 - 304)
Polycyclic Aromatic Hydrocarbons (n=4)	1,470 (49-4,670)
Phthalates (n=5)	1,760 (550-3,140)

n= the number of chemicals tested with the contact test in the study.

Table 7. Earthworm LC_{50} contact test values and rat, oral LD_{50} values for the substituted aromatics tested.

Chemical	Contact Test LC_{50}, $\mu g \cdot cm^{-2}$	Rat, Oral LD_{50}, $mg \cdot kg^{-1}$
Carbaryl	14	250
Nitrobenzene	16	640
1,2-Dichlorobenzene	21	500
1,2,4-Trichlorobenzene	27	756
Chlorobenzene	29	2,910
Ethylbenzene	47	3,500
Toluene	75	5,000
Benzene	98	3,800
Hexchlorobenzene	non-toxic up to 1,000 $\mu g \cdot cm^{-2}$	10,000

Table 8. Impact of the chemicals in the artificial test and rat, oral LD_{50} values.

Chemical	Artificial Soil Test LC_{50}, $mg \cdot kg^{-1}$	Rat, oral LD_{50}, $mg \cdot kg^{-1}$
Chloroacetamide	26	70
4-Nitrophenol	38	350
2,4,6-Trichlorophenol	58	820
Carbaryl	106	250
N-Nitrosodiphenylamine	151	1,650
Fluorene	173	NF[1]
Phenol	401	414
2-Chloroethyl vinyl ether	740	250
Dimethyl phthalate	3,160	6,900
1,2-Dichloropropane	4,240	1,900

NF - data not found

A comparison of the relevant factors associated with the contact and artificial soil tests (Table 9) identifies the advantages and disadvantages of both methods. The contact test is more rapid and less expensive to run than the artificial soil test. The artificial soil test may be more representative of what really happens to earthworms in soil ecosystems since this test permits both skin contact and ingestion of the test chemical. Neither test lasts long enough to evaluate growth and reproductive effects.

The comparisons of relative order of toxicity (Tables 6, 7 and 8) indicated that nitro-substituted compounds were generally more toxic than chloro-substituted compounds, which in turn were more toxic than alkyl-substituted compounds. All of the substituted compounds were more toxic than the parent unsubstituted compound.

The contact test and the artificial soil test are biological assay methods that can be used to estimate the biological impact of wastes and chemicals added to the soil. Both are relatively short term methods. The contact test takes two days and the artificial soil test takes fourteen days. The length of a biological assay can affect the toxicity of a chemical. If the chemical takes a long time to affect the biological system of a worm, the contact and artificial soil tests may not identify the true toxicity. An example could be Cd, which in both the contact and artificial soil tests was not as toxic as Cu, Zn or Ni. However, in our other studies (Malecki et al., 1982), in which the same five metals were analyzed for their effect on growth and reproduction, Cd was the most toxic of the five metals tested.

SUMMARY

The contact test and artificial soil tests are designed to evaluate the biological impact of potentially toxic chemicals in wastes applied to soils. Four salts (acetate, chloride, nitrate and sulfate) of five metals (Cd, Cu,

Table 9. A comparison of the parameters involved with the contact and artificial soil tests.

Parameter	Contact Test	Artificial Soil Test
Time	Short-2 days	Long-14 days
Cost	Lower	Higher
Mode of impact	Topical	Topical and ingestion
Usefulness	For compounds that pass through skin membranes	For low volatility compounds
Complexity of test	Fewer components to procedure	More components to procedure

Ni, Pb and Zn) were evaluated using the contact test. The different water soluble salts of each metal did not indicate any difference in toxicity to the worms. The order of toxicity for the metals in the contact test, from most toxic to least toxic, was Cu • Zn • Ni = Cd • Pb. The order of toxicity for the metals in the artificial soil test, from most toxic to least toxic, was Cu = Zn = Ni • Cd • Pb.

A number of organic chemicals were tested for potential toxicity using both the contact test and artificial soil test. The results were compared to available information on the rat oral LD_{50} values for the same compounds. The comparison indicated similarity between the relative toxicity of the chemicals to the worm and the rat. The chemical that was most toxic to the worm was most toxic to the rat and the chemical that was least toxic to the worm was least toxic to the rat.

The same relative toxicity was apparent for the six major chemicals groups that were tested. The two most toxic chemical groups to the worms, phenols and amines, were also the most toxic to the rat and the two least toxic chemical groups to the worms, polycyclic aromatic hydrocarbons and phthalates, were the two least toxic chemical groups to the rat.

The earthworm contact and artificial soil tests are useful in predicting the potential biological impact of organics and inorganics in wastes that may be applied to land for treatment and disposal.

ACKNOWLEDGEMENTS

This work was supported by grants from the National Science Foundation, "Waste Management Using Earthworms - Engineering and Scientific Relationships", project 80-16764, and the U.S. Environmental Protection Agency, "Earthworms as a Bioassessment Tool", cooperative agreement, CR-810006.

REFERENCES

EEC Guidelines for the Testing of Chemicals. 1982. The Contact and Artificial Soil Tests. European Economic Community (EEC) 79/831, Rev. 3.

Hartenstein, R., Neuhauser, E. F. and Kaplan, D. L. 1979. Oecologia, 43, 329-340.

Kaplan, D. L., Hartenstein, R., Neuhauser, E. F., Malecki, M. R. 1980. Soil Biol. Biochem., 12, 347-352.

Litchfield, J. T. and Wilcoxon, F. 1949. Journal of Pharmacology and Experimental Therapeutics, 96, 99-113.

Malecki, M. R., Neuhauser, E. F. and Loehr, R. C. 1982. Pedobiologia, 24, 129-137.

Neuhauser, E. F., Kaplan, D. L., Malecki, M. R. and Hartenstein, R. 1980. Agricultural Wastes, 2, 43-60.

Neuhauser, E. F., Hartenstein, R., and Kaplan, D. L. 1980. Oikos, 35, 93-98.

Neuhauser, E. F., Malecki, M. R. and Loehr, R. C. 1983. Hazardous and Industrial Solid Waste Testing (Second Conference). ASTM STP 805, American Society for Testing and Materials, 313-320.

Neuhauser, E. F., Malecki, M. R. and Loehr, R. C. 1983. Proceedings of the 37th Purdue Industrial Waste Conference, Ann Arbor Science Publishers, Inc., 253-258.

U.S. Environmental Protection Agency. 1979. "Water-Related Environmental Fate of 129 Priority Pollutants". EPA-440/4-79-029, Washington, D.C.